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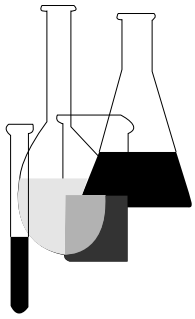
Ecological Effects Test Guidelines

OPPTS 850.4000 Background—Nontarget Plant Testing



"Public Draft"

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 850.4000 Background—nontarget plant testing.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP 120–1 Overview, 120–2 Definitions, 120–3 Basic Test Standards, and 120–4 General Evaluation and Reporting Requirements (Pesticide Assessment Guidelines, Subdivision J—Hazard Evaluation; Nontarget Plants) EPA report 540/09-82-020, 1982.

(b) **Introduction**—(1) **General.** This guideline provides general information and overall guidance for OPPTS 850, Group D—Nontarget Plants Test Guidelines. Series 850 deals with data submittal to support registration of all outdoor use pesticides that come in contact with plants and addresses testing for adverse pesticidal effects to nontarget plants, including those which are within the pesticide application target area (such as crop plants which are growing with weeds or are hosts for insects and disease organisms), and those which are outside the target area (such as typical adjacent crop plants, desirable ornamentals, garden plantings, important wildlife food and cover species, and forestry, lumber, and conservation plantings and endangered and threatened plant species). Series 850 addresses plant toxicity with respect to that resulting from either direct exposure (i.e. application of a pesticide to a plant) or from indirect exposure (i.e. exposure resulting from movement of the pesticide through the environment as from runoff, soil erosion, spray drift, etc.).

(2) **Purpose.** The purpose common to all tests is to provide data which will be used to determine the need for (and support the wording for) precautionary labeling or other statements to minimize the potential adverse effects to nontarget plants. Generally, the registrant will provide adequate precautionary labeling with respect to nontarget plants such as crops, ornamentals, and the like. However, there may be situations where the Agency will have to develop additional precautionary labeling. For example, the spraying of herbicides may not be permitted in the vicinity of critical habitats of endangered or threatened plants listed by the Department of Interior.

(3) **Organization.** (i) This group of guidelines contains two broad areas of testing procedures:

(A) Toxicity to plants in the target area.

(B) Toxicity to plants outside of the target area.

(ii) These data should be derived from tests and reported in a manner which complies with the general test standards and the general reporting requirements contained in this guideline as well as the specific standards

and reporting requirements of each guideline in OPPTS Series 850, Group D, Nontarget Plants Test Guidelines.

(c) **Definitions.** Terms used in this guideline have the meanings set forth in FIFRA at Part 162.3, section 3 regulations, and OPPTS guideline 810.1100 (for target area phytotoxicity testing). In addition, for the purposes of this guideline group, the following definitions apply:

Algae includes all chlorophyllous *Thallophyta* other than the *Bryophyta*. It includes the blue-green algae (*Cyanobacterium* or *Cyanophyta*), green algae (*Chlorophyta*), golden algae and diatoms (*Crysophyta*), brown algae (*Phaeophyta*), red algae (*Rhodophyta*), and golden-green algae (*Xanthophyta*).

Aquatic plants includes those plants that are totally aquatic (free-floating or attached, submersed and immersed) and those which are semiaquatic such as swamp and wetland plants.

Axenic is a culture of *Lemna* fronds free from other organisms.

Colony is an aggregate of mother and daughter fronds attached to each other.

Desirable plants are those plants that are not to be detrimentally affected during pesticide application. They may include crops, ornamentals, or wild plants inside or outside of the area of intended application.

ECX is the external pesticide concentration required to cause a detrimental change or alteration (in a nontarget plant) expressed as a percent (X) in comparison to untreated control plants. EC05, EC25, and EC50 are the concentrations required to effect a 5, 25, and 50 percent detrimental change, respectively, on nontarget plant growth or activity.

Endpoints is a measurement during or at the end of a test, or calculated from test data, that may be used to assess the effects of a pesticide on the test organism such as numbers of organisms that survive, percent emergence, visual phytotoxicity, growth rate measurements like plant height, plant dry weight, root dry weight.

Frond is a single *Lemna* leaf-like structure.

Frond mortality are dead fronds which may be identified by a total discoloration (yellow, white, black, or clear) of the entire frond.

LOEC is the lowest test concentration of a material used in this test that has an adverse effect.

Microorganism is any of those organisms classified as algae, fungi (*Myxomycota* and *Eumycota*), and bacteria (*Schizomycota*).

NOEC is the highest test concentration of a material used in this test that does not have an adverse effect.

Nontarget plant and nontarget microorganism are any plant and microorganism species not considered to be pests in the location in which they are growing. These species are not intended to be controlled, injured, killed, or detrimentally-affected in any way by a pesticide. Nontarget plants include desirable or pest host plants such as crops or ornamentals within the target area, and desirable plants outside the target area.

Pest-free is as free of pests as reasonably possible. For all pesticide phytotoxicity tests, damaging insects and surrounding weeds should be controlled so that healthy desirable plants are available for testing. With this action detrimental effects can be attributed to the pesticide in question, not to another pesticide, or to weeds, or damaging insects.

Phytotoxicity or plant toxicity are unwanted detrimental deviations from the normal pattern of appearance, growth, and function of plants in response to pesticides and to other toxic chemicals that may be applied with the pesticide. The phytotoxic response may occur during germination, growth, differentiation, and maturation of plants, and may be of a temporary or long-term nature. Phytotoxic responses include adverse effects on growth habit, yield, and quality of plants or their commodities to the extent that a relationship between cause and effect can be established.

Plants comprise vascular and nonvascular plants, algae, and fungi.

Representative end-use product is a pesticide product that is representative of a major formulation category (e.g. emulsifiable concentrate, granular product, wettable powder) and pesticide group (e.g. herbicide, fungicide, insecticide, etc.) and contains the AI of the applicant's product.

Semiaquatic plants are plants living in transition areas between aquatic and dry land areas, e.g., swamps, wetlands.

Static-renewal test is a test method in which the test solution is periodically replaced at specific intervals during the test.

Target area is the area intentionally treated with a pesticide when label use directions are followed.

Target area plants are all plants located within the target area, and includes both desirable and undesirable species.

TEP is a typical end-use product.

TGAI is a technical grade active ingredient.

Terrestrial plants are plants that do not require saturated soils for growth.

(d) **Nontarget area phytotoxicity testing**—(1) **Data requirements.** Data concerning the determination of outdoor pesticidal effects on nontarget area plants are required for use in ecological risk assessment. (See 40 CFR 158.150.) These data are also of use in assessments of potential off-target injury to endangered and threatened plant species listed by the Fish and Wildlife Service, Department of Interior, and when phytotoxicity concerns arise from incidents or during Special Review.

(2) **Testing scheme.** Tests in the lower tiers (Guidelines 850.4100 and 850.4400 for Tier I and 850.4200 and 850.4400 for Tier II) are designed to screen chemicals to determine the potential to cause adverse affects on seedling emergence, vegetative vigor, and aquatic plant growth and reproduction. The minimal phytotoxicity data set in Tier I applies to registrations of all pesticides except herbicides, desiccants, defoliants, and plant regulators. These tests apply to all terrestrial, aquatic, and forestry uses so that the Agency can assess the potential for short and long term adverse impacts on terrestrial and aquatic ecosystems and systematically evaluate each pesticide for potential adverse effects to endangered or threatened species. Tier II provides for generation of dose-response testing for outdoor uses of all known phytotoxicants, including, but not limited to herbicides, desiccants, defoliants, plant growth regulators and any fungicides, insecticides or other chemicals tested in Tier I which demonstrate phytotoxicity. In addition, Tier III (Guidelines 850.4300 and 850.4450) is designed to broaden the knowledge concerning any detrimental effects on nontarget plants. Progression to Tier III would occur as needed to evaluate appropriate risk mitigation methods. The criteria to proceed from one tier to the next are given in 40 CFR 158.540.

(3) **Waivers.** Waivers of specific nontarget phytotoxicity test data or protocols may be requested. The request for waiver must address the product application methodology, the pesticide product's biological, chemical, and physical properties, and the known phytotoxic properties of the pesticide product.

(4) **Substitutions.** If the pesticide or the active ingredient (AI) of the pesticide, e.g. herbicide or other phytotoxic pesticide, has been extensively tested using screening tests or other evaluation systems that are similar in intent to any tests of Tier I, the data from those tests may be submitted in lieu of the required data. The term ''extensively tested'' means dose response testing of at least the plants or plant families represented in OPPTS 850.4100 and 850.4400 under environmental conditions suitable to determine any phytotoxic effects. The reports should be submitted as provided in paragraphs (c) of OPPTS 850.4100, 850.4400, 850.4200, 850.4300, and 850.4450. In addition, paragraph (h) of this guideline lists the information to be provided regarding the plant screening data and the documentation to be provided on testing procedures. The Agency will reserve the right to require testing as provided in Tier I if the submitted

test data do not prove to be adequate to assess a pesticide's phytotoxic nature.

(e) **Target area phytotoxicity testing waiver of requirements.** (1) It has been determined that product performance test data include target area phytotoxicity testing data (see Guideline 850.4025), and that data submittals for such testing may be waived by the authority of the Administrator, under FIFRA (U.S. Code 7, 136, 3(c)(5), for most kinds of pesticide products. Such products generally include all pesticides whose uses result in direct or indirect application to plants in the target area such as rangelands and nonagricultural areas.

(2) Even though the Administrator will ordinarily waive the requirements for submittal of target areaa phytotoxicity test data as indicated in paragraph (d)(1) of this guideline, the Administrator reserves authority to require such data on a case-by-case basis, whenever the Administrator deems that such data are necessary to evaluate the acceptability of a product.

(f) **Basic test standards**—(1) **Purpose.** This paragraph contains test standards that apply to all studies in this series of guidelines. If a specific test contains a standard on the same subject, that specific test standard should take precedence in the performance of that particular study.

(2) **General.** The experimental design, execution of the experiments, classification of the organism, sampling, measurement, and data analysis in support of an application for registration must be accomplished by use of sound scientific techniques recognized by the scientific community. The uniformity of procedures, materials, and reporting must be maintained throughout the toxicity evaluation process. Refinements of the procedures to increase their accuracy and effectiveness are encouraged. When such refinements include major modifications of any test procedure or standard, the Agency should be consulted before implementation. All references supplied with respect to protocols or other test standards are provided as recommendations.

(3) **Personnel.** (i) All testing and evaluation must be done under the direction of personnel who have the education, training, and/or experience to perform the testing and evaluation in accordance with sound scientific experimental procedures.

(ii) To help assure consistency in the development of data, one person should be responsible for each particular phase of the study.

(4) **Test Substance.** (i) Use of a TEP instead of a TGAI is preferred for all terrestrial nontarget plant phytotoxicity tests. Aquatic plant studies may be conducted using the TEP or TGAI. If an adjuvant is recommended on the product label, representative adjuvants must be included in the test at the recommended dosage. The TEP selected for testing should be the

one with the highest percentage AI and/or the one most widely used. TEP's that contain other AI's should be avoided or tested separately. The use of TEP testing should eliminate the need for a separate solvent control as the solvents will already be contained in the formulation. An untreated (negative) control is still required. If a carrier, vehicle, or adjuvant is used to dissolve, dilute, or modify the physical characteristics of the test substance for any study, it should not:

(A) Interfere with the metabolism (degradation) of the test substance.

(B) Alter the chemical properties of the test substance.

(C) Produce physiological or toxic effects to plants.

(ii) In addition to or instead of data required by this guideline, the Agency may require, after consultation with the applicant, data derived from testing with:

(A) The technical grade of an AI.

(B) A contaminant or impurity of an active or inert ingredient.

(C) A metabolite or degradation product of an active or inert ingredient.

(D) A different pesticide formulation (TEP).

(E) Any additional substance which enhances the phytotoxic activity (up to and including synergistic effects) of the product for which registration is sought.

(F) Any combination of the test substances listed.

(5) **Nontarget plant test species.** (i) The organism species or groups to be tested are specified in OPPTS 850.4100 through 850.4450.

(ii) Healthy plants must be used.

(iii) Either cultivated crop, ornamental, or wild indigenous plants may be used; endangered or threatened species as determined by the Endangered Species Act of 1973 (Public Law 93–205) are not to be used. When selecting plant test species other than corn, soybean, and a root crop, the Agency encourages the use of sensitive plants other than crop plants—weeds, native species, perennial species, etc. The Agency also encourages testing of more than 10 species.

(iv) Test organisms that are obtained from natural systems and which are to be used for testing should be maintained under conditions similar to their natural or normal cultural environment.

(v) The population size of each replicate or treatment should be large enough to assure meaningful results. Sample sizes should be selected

which will yield results that are statistically significant at the 90 to 95 percent level of confidence with a significance level of less than 0.10. The sample size for each plant species in the tier tests should be of sufficient size to support the 25 or 50 percent (EC25 or EC50) progression criteria statistically.

(6) **Nontarget organism safety.** While performing field tests, all necessary measures should be taken to ensure that nontarget plants and animals, especially endangered or threatened species, will not be adversely affected either by direct hazard or by impact on food supply or food chain.

(7) **Controls.** Control groups are used to assure that effects observed are associated or attributed only to the test substance exposure. In phytotoxicity evaluations, all treated plots, plants, and commodities must be compared directly to untreated control plots, plants, and commodities. The appropriate control group should be similar in every respect to the test group except for exposure to the test substance. Within a given study, all test organisms including the controls should be from the same source. To prevent bias, a system of random assignment of the test plants to test and control groups is required. Where a carrier, vehicle, or adjuvant other than water is used, appropriate experiments and controls should be included to distinguish the possible action of the carrier, vehicle, or adjuvant.

(8) **Equipment.** (i) All equipment used in conducting the test, including equipment used to prepare and administer the test substance, and equipment to maintain and record environmental conditions, should be of such design and capacity that tests involving this equipment can be conducted in a reliable and scientific manner. Equipment should be inspected, cleaned, and maintained regularly, and be properly calibrated.

(ii) The application equipment used in testing products in small field plot studies should be designed to simulate conventional farm equipment. This can be accomplished by using the basic components of commercial application equipment in the design of the small-plot equipment. For example, nozzle types, sizes, and arrangements on small plot sprayers can be identical to those used by growers on commercial ground sprayers. Single-row commercial granular application equipment mounted on a garden tractor for small plot trials should produce results comparable to a multiple of such units on a large tractor. For large-scale field trials, commercial application equipment should be used. Specific details as to descriptions of equipment design, adjustment, and operation should be provided in test reports.

(g) **Evaluation and reporting requirements**—(1) **General.** (i) Experimental use permits may be required for the terrestrial testing of pesticides under field conditions involving more than 10 acres such as in studies described in OPPTS 850.4025 and 850.4300. A permit may be required for aquatic field testing of pesticides of more than 1 acre.

(ii) The report should include a detailed and accurate description of test procedures, materials, results, and analysis of the data, a statement of conclusions drawn from the analysis, and a tabular summary and abstract of results. When they have been determined, the primary and secondary modes of action with respect to plant morphogenic and biochemical levels should be reported.

(iii) The metric system should be used in test reports. The U.S. Standard Measures may be used to preclude extensive conversion to the metric system. The two systems cannot be mixed (e.g. grams per square feet).

(iv) The English language must be used in all test reports. English translations must be provided with foreign language reports.

(2) **Test materials and methods**—(i) **Dates.** Report the actual dates of the studies including dates of initiation (planting, transplanting, and cultural practices), applications, observations, and harvest.

(ii) **Laboratories.** The names of the laboratories or institutions performing the tests should be included.

(iii) **Personnel.** Name and title of each investigator, and the name, address, and phone number of the employer must be reported.

(iv) **Test substance.** Identification of the test substance must be provided, including:

(A) Chemical name, molecular structure, and qualitative and quantitative determination of its chemical composition.

(B) Relevant properties of the substance tested, such as physical state, pH, and stability.

(C) General identification and composition of any vehicles (e.g. diluents, suspending agents, and emulsifiers) or other materials used in the testing of the substance.

(D) Appropriate portions of this reporting requirement may be satisfied by cross-referencing to OPPTS Series 830 (Product Properties Test Guidelines).

(v) **Untreated control (check) plots.** Detailed descriptions of plots and plants used as controls for comparisons of toxic effects should be included for each test. Untreated control (check) plots should be treated and evaluated in the same manner as the treatment plots with respect to other pesticides or chemical (fertilizers, etc.) and cultural practices.

(vi) **Test organisms.** The description should include the identification of the test organisms (genus, species, and cultivar or variety, as appropriate), rationale for selection of the species employed, and location of plant collection areas including their physiographic data. When plant spe-

cies other than those identified for specific studies have been tested, their degree of susceptibility to the pesticide should be included in the test report. This susceptibility should be reported in terms of EC values as in the regular test plant reports.

(vii) **Location.** Geographic location, including relation to the target sites, should be reported.

(viii) **Substrate conditions.** (A) For aquatic pesticide applications, the following physiographic conditions should be reported:

(*1*) Type of aquatic site, such as lake, pond, reservoir, stream, or irrigation ditch with flow rate (if moving water).

(*2*) Size (area and depth or volume or length, width, and depth of the treated areas, and of the whole site), as is appropriate to the type of application and the type of target organisms.

(*3*) Water quality, including pH, temperature, hardness, alkalinity or salinity, where possible.

(*4*) Turbidity (visual), conductivity (if possible), and dissolved oxygen (for submerged plants only).

(*5*) Soil texture, including that of soils along the immediate shoreline or ditchbank and the submerged soil where the target pests are present (with the percent organic material in the soil also reported). (Recommended methods and soil texture classifications may be found in paragraph (i)(3) of this guideline.

(B) For terrestrial pesticide applications, the following physiographic conditions should be included:

(*1*) The edaphic conditions and characterization including soil type and texture, approximate pH and temperature, and K_d, and K_{ow} values.

(*2*) Where the presence of a fragipan or shallow bedrock may lead to restricted leaching or soil waterflow, the depth of that restriction.

(*3*) The degree and direction of slope and its orientation to the row direction if the slope will lead to excessive runoff.

(ix) **Environmental conditions.** (A) For growth chambers and laboratory experimentation, the light quality, light quantity (lux), air temperature, humidity, photo- and thermoperiods, and watering schedules should be reported.

(B) For greenhouse and field experiments, the approximate light quantity (usually expressed in degree of cloudiness), high and low daily air temperatures, relative humidity, and photoperiod (day length) should be reported. The environmental conditions of the specific field site are

required only for the day of application. Area or specific field environmental conditions may be used for long term studies. Rainfall is to be reported for the duration of field experiments.

(x) **Application**—(A) **General.** The test substance application method should be reported, including dosage rates, application equipment (nozzle, orifice, pressure), time and number of applications with reference to season and stage of growth), spray dilution, spray volume per unit area, and adjuvants.

(B) **Application rates.** Dosages should be reported in units of AI or acid equivalent as appropriate. Rates may be expressed as units of ingredient per unit of land area to be treated, units of concentration (such an parts per million), units per flow rate, or units of ingredient per unit volume applied to obtain a specified degree of foliage coverage (such as to runoff). If a product is applied more than once within a year or growing season, each rate and the interval between applications should be indicated. If products are applied in a tank mixture or are applied serially, rates and intervals, as appropriate, should be reported with identification and formulation for each product.

(C) **Timing of applications.** When the test substance, particularly a herbicide, plant regulator, desiccant, or defoliant, is applied to any desirable nontarget plants within or adjacent to the target area, the stage of growth or development of the plants at application should be described in test reports.

(D) **Serial applications.** In addition to the detrimental effects of the pesticides, the times of application (or application interval) should be indicated for each product or tank mix involved in the serial application.

(3) **Observations.** (i) Observations should be reported to include all variations, either inhibitory or stimulatory, between the treated test organisms and the untreated control test organisms. Such variations may be phytotoxic symptoms (chlorosis, necrosis, and wilting), formative (leaf and stem deformation) effects, and/or growth and development rates. Observations should include the stage of development and dates when adverse results occurred and subsided or recovered. Any lack of effects by the pesticide should also be reported.

(ii) Observations should be reported in sufficient detail to allow complete evaluation of the results. This evaluation, to be performed by the registrant, should include the degree or extent of effects exerted by the pesticide in question for each replicate and variable.

(iii) The detrimental or adverse effects to be considered and reported during the observation period of terrestrial studies include:

(A) Stand or plant population.

(B) Overall vigor of the plants expressed as height, weight, diameter, length, or other similar aspect of growth.

(C) Phytotoxicity or visible symptoms such as discoloration, malformation, desiccation, or defoliation.

(D) Lodging of plants.

(E) Effect on root growth and structure.

(F) Development delay or acceleration with respect to maturation.

(G) Yield of the crop or commodity that is treated as compared to those of crops or commodities of untreated check plots.

(iv) Where pesticides are applied to aquatic systems and influence plant growth and development in aquatic systems, the effects of that pesticide on nontarget plants in the system and along the immediate border should be evaluated and reported, including vigor of the plants, phytotoxicity or other visible symptoms, and delay or acceleration with respect to vegetative growth, flowering or sporulation, and maturation.

(v) Uniform scoring procedures should be used to evaluate the observable toxic responses.

(vi) At least two methods of evaluation (such as quantitative and qualitative determinations) should be used in the evaluation of pesticide effects on growth, reproduction, and yield of plants in greenhouse and controlled chamber experiments. When direct measurements cannot be made, such as in large field evaluations, a 0 to 100 or 0 to 10 rating scale should be used, where 0 indicates no injury and 100 or 10 indicates a total effect or kill produced by the test substance. An explanation of the steps of the rating scale employed should be included with the report.

(vii) Observation reports should include the basic data to be used for the statistical analysis (see paragraph (g)(4) of this guideline). Such data should include the actual values used to determine any percentages of effects. Raw data (chromatographs, field reports, and analysis data) may also be included to substantiate the basic data that are required.

(4) **Statistical analysis.** (i) When test results such as efficacy, phytotoxicity, or yield indicate adverse effects on crops and other nontarget test organisms, statistical analysis is required in the evaluation the responses. The statistical analysis should consist of:

(A) The tabulation of the response data at each treatment level.

(B) The determination of 25 or 50 percent detrimental effect levels (e.g. EC25, EC50, as appropriate) and the 95 percent confidence limits, where possible, for each.

(C) The estimated nondiscernible effect level. This is the level at which there would be no significant effect on the intended yield, quality, or aesthetics of the crop or plant which might be exposed.

(ii) Statistical analysis is also useful in evaluation of interactions resulting from studies supporting tank mixtures or serial applications.

(5) **Supporting material.** Copies of references or literature used in modifying the test protocol, performing the test, making and interpreting observations, and compiling and evaluating the results should be submitted. Copies of unpublished literature should also be included. Copies of the recommended literature referenced in these guidelines are not required.

(6) **Special test requirements.** In addition to the data required in this guideline, data from other tests may be required by the Agency for making judgments regarding safety to nontarget plants. Such data will be required where there are special problems, such as a proposed pattern of use, mode of phytotoxic action, or a unique chemical property. Methods are usually derived from those already described or cited in other guidelines.

(h) **Reporting elements for acceptability.** (Further details are provided in each guideline.)

(1) Information to be provided regarding the nontarget plant phytotoxicity screening data:

(i) Mode of action (if available).

(ii) Common and Latin names of species tested.

(iii) Species should be tested with a minimum of five doses bracketing NOEC and EC50 (or effect at maximum label rate for species not responding).

(iv) Calculation of a dose-response curve with NOEC, EC05, EC25, EC50, slope, and CI (confidence interval) for each species.

(v) Raw data preferably in electronically readable form.

(2) Documentation to be provided on testing procedures.

(i) Application method (ppi, pre-, post-emergence).

(ii) Test substance and doses used (AI, end-use product, adjuvant used).

(iii) Indoor vs. outdoor trials.

(iv) Number of replicates per dose (minimum of three).

(v) Number of plants per dose (number of plants per pot).

(vi) Endpoints used (definition of rating scales, quantitative or qualitative precision).

(vii) Seed source, stage of the plant life cycle (seed, seedling, leaf stages).

(viii) Date and duration of testing, soil type.

(ix) Bottom vs. top watering and frequency of watering.

(x) Any other relevant information pertinent to the evaluation.

(i) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Boutin, C. et al. Proposed Guideline for Registration of Chemical Pesticides: Nontarget plant testing and evaluation. Technical Report Series No. 145, Canadian Wildlife Service, Environment Canada, pp. 1 - 91 (1993).

(2) Truelove, B., (ed). *Research Methods in Weed Science*. Southern Weed Science Society. Auburn Printing Inc., Auburn, AL (1977)

(3) U.S. Department of Agriculture. Soil Survey Manual, Handbook No. 18 (1951).